Project Title	**(Preliminary) Studies on fungicides in mushroom casing**
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Previous Reports	None
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Practical Section for Growers

This project was initially intended to be of three years duration. Due to the departure of PhD student Carolyn Riches after four months, the project was terminated at that point. This report contains the results of her work to that date. The remainder of the project will be completed under a new project number (M 41a) which will commence in October 2002 and which will run for three years. Consequently there is no information generated at this point which could be of practical use to growers

1. Introduction

The project aims to study the behaviour of the fungicides carbendazim (Bavistin DF) and prochloraz (Sporgon 50WP) in mushroom casing material.

There are very few fungicides approved for use within the British Mushroom Industry. It is thought that the effects of these are becoming limited due to resistance amongst pathogen populations, with the mushroom industry experiencing a considerable loss in production due to cobweb (*Cladobotryum* spp.) and dry bubble (*Verticillium fungicola*). A major problem may be the short persistence of the fungicides in mushroom casing, so that available concentrations are low when threats to diseases are at their highest.

1.1 Project Objectives

- To determine the factors that control fungicide persistence in mushroom casing.
- To identify the potential of manipulating such factors to maximise fungicide levels throughout cropping.
- To see if the manipulation of fungicide persistence can enhance the efficacy of products under cropping conditions.

1.2 Preliminary Investigation

An initial experiment was set established to examine the effect of temperature on the rate of fungicide degradation in mushroom casing. This was achieved by using standard laboratory techniques to compare the temperatures of 18°C and 25°C over a period of 42 days. The temperatures and experimental duration were determined by mushroom cultivation conditions at HRI Wellesbourne. Fungicide residues were measured at regular intervals using HPLC analysis.

The mushroom production time-scale at HRI is summarised in Table 1.2.

Table 1.2

Day 0 - 8	• Apply prochloraz and carbendazim as mix into casing. • Apply casing: Temp 25°C, RH 90% • Heavy watering (Drench) at three time points
Day 3	Apply carbendazim and prochloraz in 2nd watering, if not mixed with casing
Day 7	Apply carbendazim and prochloraz in last watering if not applied at 2nd or in casing mix
Day 8 - 11	Gradual reduction in Temp and RH to: Temp 18°C, RH 85%. • Held to Day 40. • Known as airing (pinning-up) • Can be manipulated
Day 17	Mushrooms first appear
Day 17 - 20/21	1st flush mushrooms picked
Day 21/22	• Heavy watering (drench) • 2nd application Prochloraz at 2nd watering if first applied on Day 3
Day 25 - 28	2nd flush mushrooms picked
Day 29 - 30	• Heavy watering (drench) • 2nd application Prochloraz in 2nd watering if first applied on Day 7
Day 34 - 40	3rd flush mushrooms picked
Day 40 onward	• Cook-out (with steam) • Sterilise growing media • Clean and disinfect whole tunnel • Temp of compost raised to 65 - 70°C and maintained for 6hrs.

2. Materials and Methods

2.1 Casing Material

Casing used in the experiment at HRI was purchased from Tunnel Tech Ltd. The blend used was their English mix containing wet black peat and sugar beet lime.

The casing material was placed in exact 20 g quantities into 125 ml wide mouth jars with screw-on lids (supplied by BDH). On incubation, the lids were loosely placed on the jars and moisture content was maintained.

2.2 Fungicide Application

The fungicide formulations (Bavistin DF and Sporgon 50WP) were applied to individual jars at the commercial application rate, following label recommendations. Details of the fungicides are summarised in Table 2.2.i.

Table 2.2.i

Fungicide	Active Ingredient (a.i)	% Active Ingredient
Bavistin DF	Carbendazim	50%
Sporgon 50WP	Prochloraz manganese	46 % W/W

As can be identified from Table 1.2, mushrooms are cultivated at two temperatures; 25°C and 18°C. The relatively short harvest interval of prochloraz (48 hours) allows it to be applied in two stages, namely at Day 0 and Day 21. Carbendazim has a harvest interval of 14 days and is applied at Day 0 only.

The experiment was designed to look at three treatments for carbendazim and four for prochloraz throughout a 42 day duration, taking into account the above factors. Table 2.2.ii summarises the casing treatments for each of the fungicides.

Table 2.2.ii - Fungicides applied to individual jars

Treatment	Dose Carbendazim	Dose prochloraz	Incubate at 25° C	Incubate at 18° C
One	Day 0 35mg / L	Day 0 15 mg / L	42 days	-
Two	Day 0 35mg / L	Day 0 15 mg / L	-	42 days
Three	Day 0 35mg / L	Day 0 15 mg / L	10 days	32 days
Four	-	Day 0 15mg / L **and** Day 21 15mg / L	10 days	32 days

Samples for each treatment were taken at regular time-points (following fungicide application), throughout the 42 day duration, i.e. Day 0, 3, 7, 10, 14, 21, 28, 35, 42.

For each treatment time-point, three replicates were prepared. This enabled jars of the same treatment to be randomly placed in the incubator, preventing differences which may have arisen through environmental conditions.

At each time-point, replicates were either removed from the incubator and stored in a freezer (-15 C), or residues were extracted immediately.

Residues were extracted from casing (20 g) with acetonitrile (50 ml HPLC grade) by shaking for one hour. The extracts were then placed in 30 ml disposable centrifuge tubes and centrifuged for five minutes. The supernatant was placed in an HPLC vial using a disposable pipette.

Procloraz manganese and carbendazim were analysed by High Performance Liquid Chromatography, using a Spectra Physics SP8810 pump, Cecil CE1200 uv detector and a 250 x 4.6mm Spherisorb C8 column. Table 2.2.iii summarises the HPLC setting for each fungicide.

Table 2.2.iii

	Carbendazim	**Prochloraz**
Mobile phase	40% CH_3CN 30% MeOH 30% H_2O	50% CH_3CN 30% MeOH 20% H_2O
Detector wavelength	220nm	220nm
Detector absorbance range	0.2	0.2
Standard concentrations	10	10
Flow rate	1.3 ml^{-1}	1.6 ml^{-1}
Retention time	4.2 min	2.9 min
Run time	4.0 min	6.0 min
Start integration	2.4 min	3.7 min

3. Results and Discussion

3.1 Carbendazim

Figure 3.1.i shows the percentage recovery (degradation) of carbendazim with time. It is possible to identify that those samples incubated at 18°C degrade less rapidly than those incubated at 25°C. Final recoveries range from 25% to 45%.

The data for the samples incubated at 25°C for the first ten days of the study, follows that of the data obtained for the samples incubated at 25°C for the 42 days. When the temperature is reduced, it can be seen that the data shift to run parallel with that obtained for the samples stored at 18°C only. Such a trend can be more clearly identified when the log of the data is plotted, as shown in Figure 3.1.ii.

For those samples incubated at 25°C, the DT50 can be calculated to be 22.2 days, whilst for those samples incubated at 18°C, the DT50 is 34.0 days. The half-life is therefore increased by 11.8 days if incubated at 18° C.

3.2 Prochloraz

Figure 3.2.i illustrates the percentage recovery of prochloraz with time. As with carbendazim, it can be identified that a greater residue can be extracted from those samples incubated at 18°C compared with samples incubated at 25°C.

When comparing to carbendazim, however, it can be seen that the rate of prochloraz degradation is far less rapid, with approximately a 70% recovery at the end of the experiment.

Figure 3.2.ii, shows that the initial degradation of prochloraz follows that of those samples stored at 25°C. The repeated application of prochloraz can be clearly identified by a sharp increase in the percentage recovery, with residue, concentrations at 25 mg / kg equivalent to 160%. The DT50 for samples incubated at 25°C can be calculated to 96.3 days, whilst samples incubated at 18°C show a DT50 of 110.0 days. The half-life is increased by 13.7 days if stored at the lower temperature.

3.3 Summary

This initial experiment provided a good background in techniques for the study of fungicide persistence in mushroom casing, and also enabled the effect of temperature on fungicide degradation to be examined.
The results show that both the fungicides, carbendazim and prochloraz, degrade in mushroom casing with time - carbendazim concentrations falling at a greater rate. Despite fungicide concentrations falling, residues remaining at the end of the experiment are high enough to control pathogen populations.

It can be suggested that low fungicide concentrations found in mushroom casing in previous HDC[1] reports are not influenced by temperature, but that a series of factors are involved in creating the low concentrations found in mushroom casing material. An example of such a factor could be the wetting and drying regimes of the casing.

Further experimentation can therefore be aimed at the inclusion of such factors so as to eliminate the variables which affect fungicide degradation.

[1] **Grogan, H.M., Jukes, A. and Cael, N. 1999**. Fungicide profiles in casing. Final Report for HDC Contract No M30a.

Figure 3.1.i

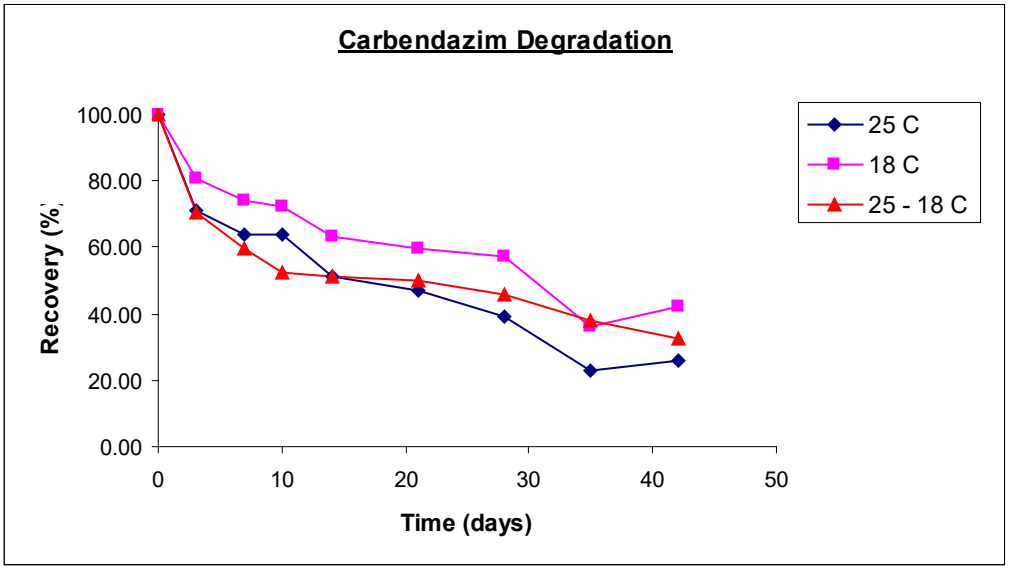

Figure 3.1.ii

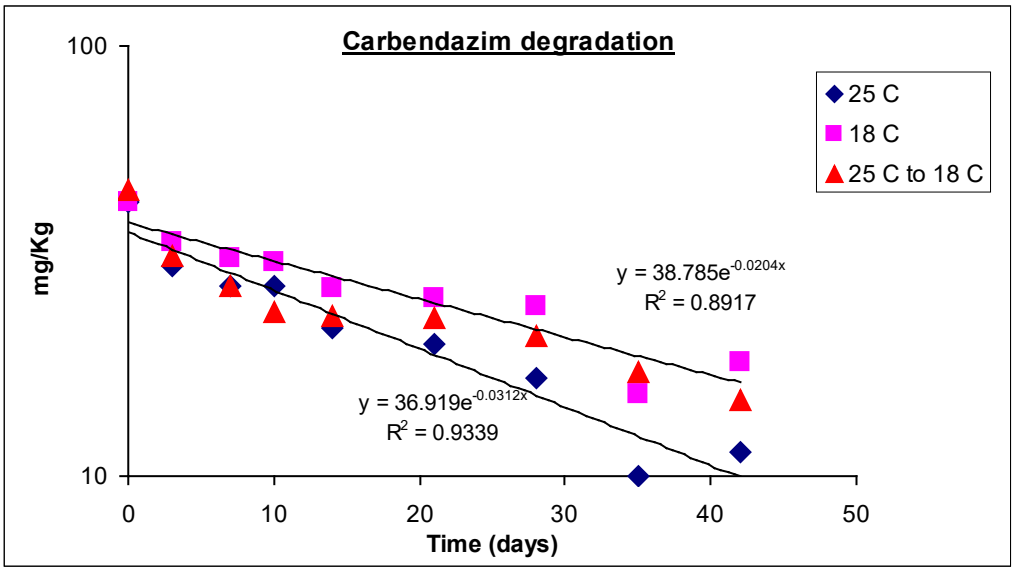

Figure 3.2.i

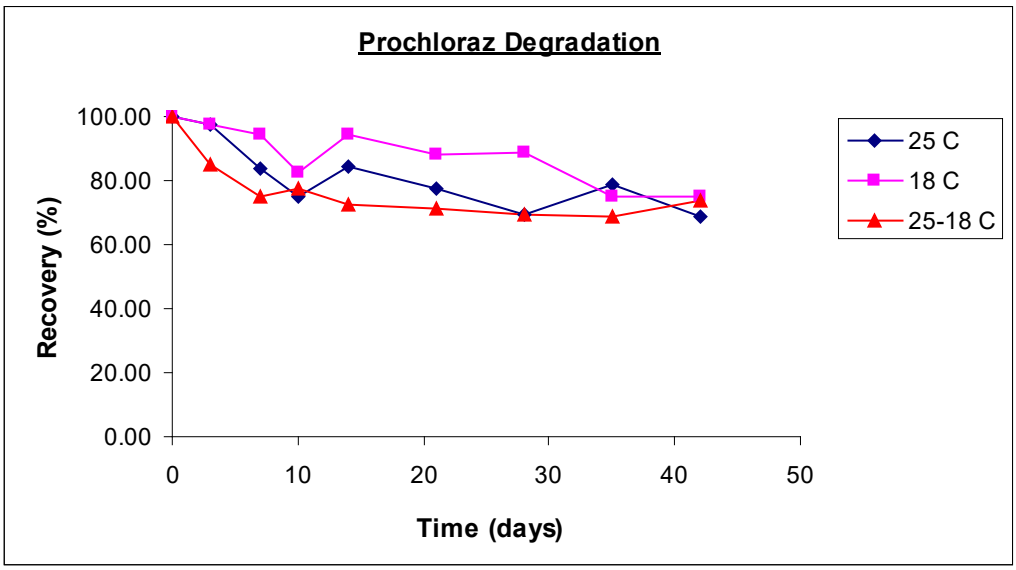

Figure 3.2.ii